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A NOVEL ELIMINATION REACTION CATALYSED BY HUMAN α -METHYLACYL-COA RACEMASE (AMACR; P504S), AND POTENTIAL APPLICATIONS FOR MEASURING ENZYME ACTIVITY.

Maksims Yevglevskis, Michael D. Threadgill, Timothy J. Woodman, Matthew D. Lloyd

Medicinal Chemistry, Department of Pharmacy & Pharmacology, University of Bath, Claverton Down,

Bath BA2 7AY, United Kingdom

Email: M.Jevglevskis@bath.ac.uk

Branched-chain 2-methyl fatty acids are derived from abundant dietary lipids and cholesterol. β -Oxidation of these lipids requires *S* stereochemistry, but those with *R* stereochemistry are common. α -Methylacyl-CoA racemase (AMACR; P504S) catalyses chiral inversion, enabling subsequent metabolism. The enzyme is also important for conversion of the *R*- enantiomers of ibuprofen and related drugs to their pharmacologically active *S*- enantiomers. The enzyme works by a deprotonation/reprotonation mechanism via an enolate intermediate.¹

AMACR protein levels and activity are increased in all prostate cancers. Reduction of AMACR levels using siRNA causes prostate cancer cells to revert to a more normal phenotype, including restoration of androgen dependent growth. The enzyme is therefore a promising new drug target. However, development of this target has been hampered by the difficulties in measuring chiral inversion and the resulting lack of a convenient, high-throughput assay. Consequently, only three studies on inhibitors of AMACR have been reported since 2001.

Incubation of novel substrates containing fluorine with active human recombinant AMACR 1A resulted in the formation of an unsaturated product. Negative controls containing inactive enzyme did not produce a significant level of unsaturated product. Incubation of the unsaturated product and fluoride with active enzyme did not result in the formation of substrate, showing that the elimination reaction was irreversible. Steady-state kinetic analysis of the reaction shows that the elimination of fluoride from this substrate is *ca.* 32 x more efficient than chiral inversion of *S*-2-methyldecanoyl-CoA, as judged by k_{cat}/K_m . This new reaction has the potential to lead to the development of a high-throughput assay for discovery of AMACR inhibitors and may also aid the development of more convenient techniques for diagnosing and monitoring prostate cancer.

References

1. M. D. Lloyd, M. Yevglevskis, G. L. Lee, P. J. Wood, M. D. Threadgill and T. J. Woodman, *Prog. Lipid Res.*, 2013, 52, 220-230.